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GENES FOR RUST RESISTANCE IN WHEAT

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PROJECT 20000251 – GENES FOR RUST RESISTANCE IN WHEAT

A. ABSTRACT

Leaf and stem rust of wheat are an ever-present threat. The purpose of this project is to provide wheat breeders with the information on the inheritance of rust resistance and the sources of resistance that they need to produce resistant varieties. Rust resistant wheat varieties were obtained from many countries. Their genes for resistance were transferred singly to a susceptible genotype. The resulting lines were tested with either 13 races of stem rust or 10 races of leaf rust. Many lines that are resistant to all of the races of one type of rust have been developed. They should be valuable in wheat breeding.

B. EXECUTIVE SUMMARY

The major objective of the project is to provide wheat breeders with information on the inheritance of leaf and stem rust resistance in wheat and to develop lines carrying genes for resistance that will be useful in breeding.

1. Identification of New Genes for Rust Resistance

For many years, leaf and stem rust resistant varieties were obtained from A number of countries. Their genes for resistance to stem rust were transferred to a very susceptible bread wheat, LMPG-6, by backcrossing. For leaf rust resistance, several susceptible bread and durum wheats were used for the same purpose. To determine the usefulness of the genes, the lines carrying single genes for stem rust resistance were tested with a set of 13 stem rust races. Similarly, the lines carrying genes for leaf rust resistance were tested with a set of 10 races of leaf rust.

Initially, the work was concentrated on stem rust resistance. Infection types on a scale of 0 (immune) to 9 (fully susceptible) for 295 lines carrying single genes and 66 parents were entered on a spreadsheet. The entries were then sorted, first according to the number of races they gave resistance to (0 to 13) and then within these groups according to their patterns of resistance and susceptibility. For example, the lines having resistance to only two races could be resistant to any two of the 13 races (78 possible combinations). Lines having resistance to the same two races probably carry the same gene but this has to be confirmed by intercrossing them. Lines resistant to different pairs of races must carry different genes. The analysis of the data suggested many crosses that need to be made to confirm the tentative conclusions. Forty-nine lines were resistant to all 13 races and have the greatest potential in wheat breeding.

Similar work has been done to identify genes for leaf rust resistance. Three hundred and two lines and parents have been tested with 10 races of leaf rust and the data are ready to be entered on a spreadsheet for analysis. One interesting observation has been made. A majority of the lines are resistant to all 10 races, although the resistance is often of an intermediate level.

2. Suppressors of genes for Stem Rust Resistance

Genes that suppress the effect of genes for rust resistance have occasionally been reported. Studies on Thatcher spring wheat, Triumph 64 winter wheat and Medea durum wheat showed that they all carry suppressors, suggesting that suppressors might be much more common than previously thought. To test this, crosses were made among seven durum wheats susceptible to race 15B-1 of stem rust. The F_1 plants were susceptible but in 11 of 20 crosses the F_2 plants segregated for resistance. In these 11 crosses, at least one of the parents must carry a suppressed gene for resistance that is expressed in plants from the cross that lack the suppressor. In a similar test involving susceptible bread wheat varieties, 8 of 21 crosses segregated in the F_2 generation. Suppressors must be much more common than previously thought and there must be many genes for resistance that have not yet been identified.

3. The Transfer of Stem and Leaf Rust Resistance to Wheat from Its Relatives

Durum wheats often have good resistance to leaf rust but breeders have had difficulty transferring the resistance to bread wheat. To test this further, five resistant durum wheat varieties were crossed in all combinations with four susceptible bread wheat varieties. The hybrids were backcrossed to the bread wheat varieties. In 13 of the 20 crosses, resistance was successfully transferred to the bread wheat, although the level of resistance was often somewhat reduced.

Emmer wheats (wild durum) often carry good resistance to leaf and stem rust resistance. Eleven emmer accessions were selected and crossed to both susceptible durum and bread wheats. A number of successful transfers of genes conditioning resistance to either leaf or stem rust were made. Some of the lines are resistant to all races with which they have been tested and should be useful in wheat breeding.

4. Conclusion

The project has resulted in the production of a large number of wheat lines carrying previously unidentified genes for stem or leaf rust resistance, many of which should be valuable in wheat breeding. In addition, it has provided a substantial amount of new information about the inheritance of rust resistance.

C. TECHNICAL REPORT

The greenhouse portion of the research suffered a setback in the spring of 2003. As a result of the completion of new greenhouse facilities, more space became available in the older greenhouses that I use in my work. Over a period of about three weeks in February and March, I planted pots on seven beds. As the seedlings reached the two-leaf stage, they were inoculated with rust in the way that I have always used. Seven or eight days after the first inoculation, the seedlings were inoculated with a second race. Again this is

a procedure that I use routinely. The first two beds that were rusted showed excellent infections. From then on, little if any infection was obtained.

The first question I asked was what had happened in the period between the inoculation of the first two beds and the rest. As it happened, in that period the ultra-low temperature freezer in which the spores are stored had failed and the rust spores were exposed to normal lab temperatures. However, before the spores are used they are always tested for germination by floating them on distilled water containing a small amount of Tween-20, a wetting agent. Although the thawing seemed to have had an effect on some tubes of some races, all of the spores used in the inoculations had shown normal germination. Thus, this was not the problem.

I next checked each step of the inoculation procedure to see if something was causing a failure of spore germination. In the first step, the spores are suspended in a light mineral oil and sprayed on to the plants. Spores suspended in the oil were floated on distilled water plus Tween and germinated well. In the next step, a hand sprayer is used to mist the inoculated plants with distilled water containing Tween. Spores were floated on water from the sprayer and germinated normally. In the third step, a frame is put over the bed and covered with plastic. A mist sprayer containing distilled water and Tween is put under the plastic and left running overnight to keep the plants moist while infection is taking place. Water was removed from the two, 50-year old sprayers and spores floated on it. The problem became apparent. Although the results were somewhat variable, the spores often failed to germinate. The sprayers were cleaned thoroughly with soap and water and refilled with distilled water plus Tween. Water taken from them still prevented spore germination. Then ethyl alcohol and finally bleach were tried but the spores still would not germinate. The sprayers appear to be made of galvanized metal and I think that for some reason enough zinc started to erode from them to prevent spore germination. Heavy metals such as zinc, copper and lead are known to inhibit germination.

To overcome the problem, normal house type mist sprayers were tried. Water from them had no effect on spore germination. However, the first time that one was used in the inoculation of a bed of plants, the infection was erratic. The sprayer had run out of water during the night and some parts of the bed had apparently dried out before infection had occurred. The problems were still not over. In October, five beds of pots were planted and inoculated using the house type sprayers. The sprayers were set at a lower rate of spray so that they did not run out of water during the night. The first bed had an excellent infection. The second was fair but mildew was starting to develop. The remaining three beds were overrun by the worst mildew I have seen. Mildew is not normally a major problem in the fall. And, of course, if you are infecting plants with rust you cannot treat them for mildew since the chemical will kill the rust as well.

I am now waiting until February when growing conditions are better, to plant more seed. Unfortunately, I lost considerable time and plant material. Fortunately, greenhouse tests are only part of the project and, of course, the problem occurred only in the final year. Nevertheless, it was frustrating. Despite the problems, a substantial amount of work was accomplished during the three-year term of the grant.

1. Identification and Testing of New Genes for Rust Resistance

a) Stem Rust Resistance

Over a period of many years, wheat varieties with stem rust resistance were obtained from many sources in many countries. Their genes for stem rust resistance were transferred to a common genetic background by backcrossing to a susceptible line, LMPG-6. The latter is a day-length insensitive line that I produced specifically for the purpose and is susceptible to all races with which I have tested it. In most cases, five backcrosses were made to produce near-isogenic lines (lines carrying single genes for stem rust resistance in a common, susceptible genetic background). In addition, some genes were transferred to LMPG-6 from species related to wheat (see later section for details). Included in the lines are nearly all of the named genes for stem rust resistance (*Sr* genes). As new genes are identified and named anywhere in the world the parents are obtained and the genes are transferred to LMPG-6. Thus it is a continuous process.

When this contract started, 340 lines, including some parents, had been produced and tested with a number of stem rust races. A first group was tested with 9 races, a second with 10 and a third group with 13. A final group of 121 lines has now been tested with all 13 races. In addition, the lines that had been tested with only 9 or 10 races were tested with the remaining 3 or 4 so that all of the lines have been tested with all 13 races. In total 461 lines and parents were tested

After removing some lines that proved to be duplicates, the data for 295 lines and 66 parents were entered on a spreadsheet, i. e., the infection types for each line with the 13 races was entered. The infection types were originally classified on a standard 0 to 4 scale with pluses and minuses to indicate deviations from the norm. In order to use the computer to sort the data, the infection types were converted to a 0 to 9 scale. The lines were then sorted in two ways. First they were sorted according to the number of races to which they gave resistance, i. e., from 0 to 13. Then within each group, the lines were sorted according to their patterns of resistance and susceptibility. If two lines show the same pattern, i. e., they are resistant to exactly the same races, they may carry the same gene. However, this can be proven only by crossing the two lines and seeing if their F_2 progeny segregate for resistance. If two lines show different patterns of resistance, they must carry different genes.

The final step is to group near-isogenic lines that are related in some way such as their origin, and analyze the results. The lines have been grouped into about 30 such groups (a few may still be combined). The results are then ready for publication. Earlier I indicated that the Canadian Journal of Plant Science was interested in publishing the material, subject to normal review. I submitted an introduction to the series and the first short note. They were turned down. I think I can fairly state that the whole review process was bungled from the start. The review took almost 10 months instead of the normal 4 or 5. The Associate Editor clearly did not do his or her job – at least I received no comments from him or her. One reviewer was extremely critical but quite clearly did not have a

good understanding of what a near-isogenic line is or what a race is. As a result many of the reviewer's criticism were nonsense. Anyway, I have since talked to the editor of the Canadian Journal of Plant Pathology and explained the situation. He indicated that the Journal would be interested in the series of papers, again of course, subject to review. I have just finished revising the first two papers, taking advantage of the comments I received from the Canadian Journal of Plant Science. Although the series will be rather different than normal scientific publications, I am optimistic that they will be accepted.

The final analysis of the data has resulted in a long list of crosses that need to be made to test various hypotheses suggested by the results. Some of these have already been made but many more need to be done. Forty-nine of the near-isogenic lines were resistant to all 13 races and are the most interesting as sources of genes with potential for use in wheat breeding. Some of them may prove to carry the same gene but most probably do not. Testing this would require intercrossing the 49 lines in all possible combinations (1176 crosses) and testing their progeny, an impossible task. I have had discussions with Dr. Jan Dvorak of the University of California, Davis, about the possibility of using molecular markers to locate the chromosomes carrying the genes. He has proposed a method that should work. However, I have tested with six races the stock that he wants to use as a source of molecular markers and it was resistant to all six – it has to be susceptible to at least one race to be useful. An initial plan to begin making the crosses has had to be deferred.

For some time, I have been concerned about the eventual fate of all of my genetic stocks. Dr. T. Fetch of the Cereal Research Centre, Winnipeg, has expressed interest in preserving them and we are already doing some cooperative work. For example, we are working toward having a set of my near-isogenic lines used as the standard differential lines for identifying stem rust races in North America. In the meantime, a recent appointee in the Department of Plant Sciences, Dr. C. Pozniak, has expressed interest in the material, particular for molecular genetic studies, but also in preserving it. We will be following up on this.

b) Leaf Rust resistance

Less work has been done with leaf rust resistance but a number of near-isogenic lines have been produced, some in the LMPG-6 background and some in a durum wheat background. A set of 186 near-isogenic lines and parents had been tested with 10 leaf rust races before this contract began. Since then, tests on another 116 lines has been completed. The lines now need to be entered on a spreadsheet and sorted as was done for the stem rust lines. One interesting observation has already been made. A surprising number of the leaf rust resistant lines are resistant to all 10 races, although their resistance is often of an intermediate level.

2. Analysis of Stem Rust Resistance in Specific Varieties

Previous genetic analyses of stem rust resistance in Thatcher spring wheat, Triumph 64 winter wheat and Medea durum wheat showed that in each case the inheritance of

resistance was very complex. A paper was published on each variety. In each case it was evident that genes in addition to the ones already identified were present. New backcrosses to LMPG-6 are being made to transfer additional genes. More homozygous resistant lines are being produced, particularly for Thatcher. A field test of some F_6 lines from the cross, Thatcher/LMPG-6, showed that Thatcher carries additional genes that are effective only in adult plants (published in a short paper).

The gene *Sr6,* which is present in a number of old Kenyan varieties, has a number of unusual characteristics. It is highly sensitive to variation in light and temperature. In addition, it can be dominant or recessive depending on the cross in which it is segregating or the race of stem rust used in the tests. In 2001, I published a paper on abnormal segregations in crosses involving *Sr6* and suggested that they might be due to transposons. Transposons are genetic elements that can move from one place to another on a chromosome and upset gene expression. An extensive set of new crosses was made to test this further. Again abnormal segregations occurred, although not necessarily in the same crosses as before. The final tests on this material were spoiled by the problems with rust testing described earlier and will have to be redone.

3. Suppressors of Genes for Stem Rust Resistance

The genetic analysis of stem rust resistance in Thatcher, Triumph 64 and Medea showed that in each variety genes were present that suppressed other genes for rust resistance. Although suppressors of rust resistance have been reported occasionally, the data suggested that they might be much more frequent than previously expected. Tests were done to try and confirm this.

In the first test, seven durum wheat varieties that are susceptible to race 15B-1 of stem rust were intercrossed in all combinations except one. They were also crossed and backcrossed to LMPG-6 bread wheat. The F_1 plants from all of the crosses were susceptible to race 15B-1. However, 11 of the 20 durum crosses segregated in the F_2 generation. Thus, in these crosses at least one parent carried a gene for resistance to race 15B-1 that was suppressed and the second parent did not carry the suppressor. In the F_2 generation, some plants lacked the suppressor but carried the gene for resistance and were resistant. All seven crosses and backcrosses to LMPG-6 segregated. At least one parent in each cross must carry a suppressed gene for resistance, probably the durum parent. The genes are being transferred to LMPG-1 by backcrossing.

A similar test was done using seven common wheat varieties susceptible to race 15B-1. All six crosses involving the variety Gabo and two involving Thatcher segregated for resistance in the F_2 generation. Thus, it appears that two of the parents carry suppressed genes for resistance. Lines homozygous for the genes are being produced. In retrospect, I realized that the susceptible parents had not been well chosen. Several of them are known for their susceptibility to stem rust. It would have been better to select parents that had resistance to many of the older races of stem rust but were susceptible to race 15B-1.

The results show clearly that many varieties of wheat carry suppressed genes for resistance. Consequently, there are probably many more genes for resistance than previously realized. The results of this work have been published in the Proceedings of the Tenth International Wheat Genetics Symposium, 2003.

4. Leaf Rust Resistance in Durum Wheat

Relatively little research has been done on leaf rust resistance in durum wheat, partly because most durum wheat varieties have adequate resistance. About 15 years ago, some varieties appeared to become more susceptible and some genetic studies were started on a number of North American durum varieties. These studies have been continued and a number of genes for resistance have been transferred to a susceptible durum line, RL6079. The resulting near-isogenic lines have been tested with 10 races of leaf rust.

5. The Transfer of leaf and Stem Rust Resistance to Wheat from Its Relatives

The relatives of wheat carry genes for resistance to many wheat diseases, including the rusts. The more closely the relatives are related to durum or bread wheat, the easier it is to transfer resistance.

Many durum wheat varieties carry excellent resistance to leaf rust and are easily crossed with bread wheat. However, attempts to transfer their resistance to bread wheat have often failed, perhaps because of the presence of suppressors in the bread wheat. To test this, five resistant durum wheat varieties were crossed in all possible combinations with four susceptible bread wheat varieties. The F_1 hybrids were backcrossed to their bread wheat parents and the progeny selected for leaf rust resistance. Leaf rust resistance has been transferred to the bread wheat parent in 13 of the 20 crosses, although the degree of resistance is often less that in the durum parent. Homozygous resistant lines from two of the crosses have been tested with 10 races of leaf rust. More homozygous resistant lines have been produced and will be tested with the 10 leaf rust races.

Many emmer wheats (wild durum) also have good resistance to leaf and stem rust and are easily crossed with both durum and bread wheat. In previous work, eleven accessions of emmer wheat were selected and crossed to both susceptible durum and bread wheat lines. A number of genes conditioning resistance to either leaf or stem rust have been transferred to durum wheat and to bread wheat. Homozygous resistant lines have been tested with either 10 races of leaf rust or 13 races of stem rust, whichever was appropriate. Some of the lines should be useful in wheat breeding. A paper on the work has been submitted for publication.

C. PERSONNEL

Mr. Badrul Alam Dewan has been employed on the project part time to take care of greenhouse and growth chamber material.

D. EQUIPMENT

No equipment costing over $500 has been purchased with project funds.

E. PROJECT DEVELOPED MATERIALS

The only materials developed from the project are scientific papers. One paper has been published (copy attached), one has been submitted for publication and two are essentially ready for submission, subject to minor revision.